# Effect of Sugarbeet By-Products on the Solubility and Availability of Ferrous Sulfate in Soil

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## ABSTRACT

Ferrous sulfate solutions applied to crop or weed residues, prior to tillage and planting, have been shown to reduce iron (Fe) deficiency chlorosis of subsequent crops. The objective of this study was to determine if ferrous sulfate solutions applied with sugarbeet (*Beta vulgaris* L.) by-products, and other plant materials, would enhance Fe solubility and availability in soil. Four laboratory experiments and five field trials were conducted. In the laboratory, ferrous sulfate applied to a diversity of plant materials, prior to mixing with soil, increased the DTPA-extractable Fe in soil compared to ferrous sulfate applied alone. Sugarbeet molasses was found to be more effective in increasing Fe solubility than wheat (*Triticum aestivum* L.) straw, sunflower (*Helianthus annuus* L.) hulls, or sugarbeet pulp. Spent molasses (desugarized molasses, raffinate) was found to be as effective as regular molasses at increasing the solublility of Fe in the soil. Under field conditions, ferrous sulfate solutions applied in the fall to wheat straw, followed by tillage, increased Fe solubility in the soil over the control. Ferrous sulfate plus sugarbeet molasses applied to wheat straw, followed by tillage, gave the greatest DTPA-extractable Fe levels in soil. Iron deficiency chlorosis of soybean (*Glycine max* L. Merr.) the following year was

**slightly reduced by Fe fertilizer treatments at one of the sites, but seed yields were not improved. Further studies are warranted, to determine if solutions of ferrous sulfate plus molasses or spent molasses could alleviate Fe deficiency with other methods of application, such as broadcast and incorporated shortly before planting, or banded near the seed.**

**Additional Key Words**: iron, straw, molasses, spent molasses, raffinate, DTPA, soybean, chlorosis.

Iron deficiency of soybean is widespread on alkaline soils of the North Central region of the U.S.A. The selection of chlorosis-resistant cultivars is the most practical control measure, being more effective than foliar sprays or chelate seed treatment (Goos and Johnson, 2000a). Soybean cultivars with the greatest yield potential are not always the cultivars with the greatest level of chlorosis resistance, so the need for effective and inexpensive Fe fertilizers remains. Fertilization options are limited, due to the expense of chelates, rapid precipitation of soil-applied inorganic materials, and inconsistent or short-lived response to foliar sprays. An iron fertilization program has not been identified that is both inexpensive and effective.

Ferrous sulfate is usually the least expensive Fe fertilizer source, but ferrous sulfate is quickly rendered insoluble in alkaline soils (Goos and Germain, 2001; Sahrawat, 1988). Soil applications of ferrous sulfate are usually ineffective unless heavy rates are incorporated (Mathers, 1970) or placed in the seed row (Hergert et al., 1996). Given the low cost of ferrous sulfate compared to chelates, numerous studies have been conducted to find methods of increasing the availability of ferrous sulfate in soil. Examples include the application of ferrous sulfate suspended in liquid fertilizers (Mortvedt and Giordano, 1973), fluid suspensions of ferrous sulfate and polyacrylamide (Mortvedt et al., 1992b), dry granules of ferrous sulfate and polyacrylamide (Mortvedt et al., 1992a), or application of ferrous sulfate with acids (Mortvedt and Kelsoe, 1988).

A novel method of increasing the availability of Fe from ferrous sulfate was developed in Texas (Mostaghimi and Matocha, 1988; Matocha, 1984; Matocha and Pennington, 1982). Ferrous sulfate solutions were sprayed on crop or weed residues and tilled in. This method of application, termed "plant complexed Fe" gave a partial alleviation of Fe deficiency of sorghum (*Sorghum bicolor* L. Moench) under both greenhouse and field conditions. Applications of ferrous sulfate to pigweed (*Amaranthus* sp.) generally gave a better crop response than applications to sorghum residue. In a study in Israel (Plessner et al., 1998), ferrous sulfate treatment of plant tissue (*Azolla* sp.) produced an effective slow-release Fe source for cucumber (*Cucumis sativus* L.).

Chemically-altered sugarbeet by-products have been proposed as carriers of micronutrients, or used to remediate heavy metal-contaminated materials. Several papers (Clemens et al., 1990; Whitehurst and Clemens, 1984; Whitehurst et al., 1989) described the manufacture and properties of glucoheptonate-based micronutrient fertilizers, made from carbohydrate-rich agricultural by-products, such as sugarbeet molasses or unmarketable sweet potatoes (*Ipomoea batatas* L.). Hydrolysates of sugarbeet molasses were used to leach heavy metals out of contaminated fly ash (Bipp et al., 1998) and heavy metal-polluted soil (Fischer et al., 1998). Silage effluent made from sugarbeet tops has been used to remove heavy metals from soil (Leidmann et al., 1995).

Sugarbeet production in the U.S.A. largely occurs in areas of alkaline soils, and large quantities of sugarbeet by-products are available in many areas where Fe-deficient crops also exist. Thus, the first objective of this study was to determine if sugarbeet by-products would increase the solubility of Fe from ferrous sulfate, in soils known to produce Fe chlorosis in soybean. The second objective was to determine if the treatment of wheat straw with ferrous sulfate or ferrous sulfate plus sugarbeet molasses would alleviate Fe deficiency chlorosis in soybean under field conditions.

## MATERIALS AND METHODS

Four laboratory studies were conducted, and a field experiment was conducted at five locations in 2000. Common procedures used in all four laboratory experiments were as follows: 40 g of Ulen sandy loam (Aeric calciaquolls) were incubated in 100 mL plastic cups at 150 g kg$^{-1}$ water content at room temperature (approx. 22 °C). The cups were covered with plastic lids with four 1-mm holes for aeration. The cups were weighed weekly, and water loss by evaporation replenished. After incubation, triplicate cups of each treatment were taken, the soil transferred to 250 mL French square bottles, and extracted by shaking for 2 hours with 80 mL of the DTPA extraction solution of Lindsay and Norvell (1978). After filtration, the Fe concentrations of the filtrates were determined by atomic absorption spectrophotometry. Properties of the Ulen soil are shown in Table 1. The soil was collected from a soybean field showing severe Fe deficiency (Leonard site, Goos and Johnson, 2000a). Molasses products were used as received. Other plant materials were dried (50 °C) and ground (< 1-mm) before use.

**Table 1**. Selected soil characteristics.

| | Saturation extract † | | | | | | |
|---|---|---|---|---|---|---|---|
| Experiment | pH | EC | SAR | $CaCO_3$ | OM | Series ‡ | Texture |
| | | dS $m^{-1}$ | | g $kg^{-1}$ | g $kg^{-1}$ | | |
| Lab studies | 8.5 | 2.1 | 4.4 | 27 | 32 | Ulen | sandy loam |
| Field studies | | | | | | | |
| Argusville | 7.9 | 2.5 | 0.1 | 59 | 37 | Bearden | loam |
| Arthur | 8.0 | 0.5 | 3.8 | 37 | 26 | Wyndmere | sandy loam |
| Ayr | 7.9 | 3.4 | 1.6 | 78 | 35 | Glyndon | clay loam |
| Casselton | 8.1 | 0.6 | 0.1 | 25 | 28 | Glyndon | loam |
| Galesburg | 8.0 | 1.8 | 1.4 | 143 | 58 | Ulen | sandy loam |

† EC=electrical conductivity (salinity), SAR=sodium adsorption ratio (sodicity).

‡ All series are Aeric Calciaquolls.

### Laboratory Experiment 1.

This experiment was conducted to confirm the observations of Matocha and coworkers (Matocha, 1984; Matocha and Pennington, 1982; Mostaghimi and Matocha, 1988), that application of ferrous sulfate with plant materials increases iron solubility in soil. Twenty g of soil were placed in the bottom of the 100 mL cup, and moistened with 3 mL of water. The treatments were then placed on the soil surface. Treatments were a factorial combination of two ferrous sulfate rates (0 or 0.2 mL of a 200 g $L^{-1}$ solution of ferrous sulfate heptahydrate, giving 0 or 8 mg Fe $cup^{-1}$), four plant materials (none, soybean meal, young pigweed plants, and first-cut alfalfa, *Medicago sativa* L.) applied at 0.4 g $cup^{-1}$. The ferrous sulfate solution was either placed on the soil surface, or mixed with the plant materials, allowed to air dry, and then spread on the soil surface. An additional 20 g of soil was placed on the soil surface, to cover the treatments, followed by 3 mL of water. The cups were then capped, incubated, and analyzed after 1, 2, or 4 weeks of incubation. The rate of ferrous sulfate, on a soil mass basis, was 0 or 200 mg Fe $kg^{-1}$. The rate of plant materials, on a soil mass basis, was 10 g $kg^{-1}$. The iron content of the plant materials was not determined, but the Fe contribution of the plant materials should have been negligible.

### Laboratory Experiment 2.

Experiment 2 was performed in a similar way as Experiment 1, with 20 g of soil placed in the bottom of the cup, moistened with 3 mL of water, the experimental treatments applied, followed by 20 g of soil and the remaining water. Treatments included a control, with no ferrous sulfate or plant materials added to the soil. The second treatment was a ferrous sulfate solution (0.2 mL of 100 g ferrous sulfate heptahydrate $L^{-1}$) applied to the soil surface (4 mg Fe $cup^{-1}$ or 100 mg Fe $kg^{-1}$ of soil). Ferrous sulfate was also applied with wheat straw, confectionary sunflower shells, sugarbeet pulp, and sugarbeet molasses. The plant materials were applied at 0.2, 0.4, and 0.8 g $cup^{-1}$ (5, 10, 20 g $kg^{-1}$ of soil). The ferrous sulfate solution was mixed with the dry plant materials, allowed to air dry, and then placed on the soil surface. Ferrous sulfate was mixed with a diluted sugarbeet molasses solution, and applied to the soil surface in liquid form. The treatments were covered with 20 g of soil and 3 mL of water. The volume of the final water application was adjusted to compensate for the volume of liquid applied in the ferrous sulfate plus molasses treatments. Incubation times were 1, 2, 4, and 8 weeks. The molasses had an analysis (on an "as-received" basis) of 227 g $kg^{-1}$ water, 83 g $kg^{-1}$ crude protein, 89 g $kg^{-1}$ ash, and 547 g $kg^{-1}$ total sugars. The molasses contained 85 mg

$kg^{-1}$ total Fe. The Fe added to the soil in the molasses, on a soil weight basis, was about 0.4, 0.9, and 1.7 mg $kg^{-1}$ of soil for the three rates applied (0.2, 0.4, 0.8 g $cup^{-1}$).

### Laboratory Experiment 3.

Experiment 3 was performed to determine if the effect of plant materials on Fe solubility was improved with addition of ammonium sulfate. The experimental design was a complete factorial of two levels of a ferrous sulfate solution (0, 4 mg Fe $cup^{-1}$), two levels of an ammonium sulfate solution (0, 8 mg N $cup^{-1}$), and three plant materials (none, 0.4 g of wheat straw, or 0.2 g of molasses $cup^{-1}$). Incubation times were 1, 2, 4, and 8 weeks. Other details were the same as for Experiments 1 and 2.

### Laboratory Experiment 4.

This experiment was to compare the effects of regular molasses, spent molasses, and method of application on Fe solubility. The treatments included a control, with no ferrous sulfate or plant material added. The remaining six treatments were a factorial combination of three fertilizer solutions (ferrous sulfate, ferrous sulfate plus molasses, and ferrous sulfate plus spent molasses) with two methods of application (mixed with the entire soil mass or applied as a spot application). The fertilizer solutions were applied at a rate of 0.4 mL $cup^{-1}$ and contained 50 g ferrous sulfate heptahydrate $L^{-1}$, and 500 g molasses or spent molasses $L^{-1}$ as appropriate. The rate of ferrous sulfate was 4 mg Fe $cup^{-1}$ (100 mg Fe $kg^{-1}$ of soil) and the rate of molasses or spent molasses was 0.2 g $cup^{-1}$ (5 g $kg^{-1}$ of soil). The mixed application was made by adding the fertilizer solution with 6 mL of water, followed by addition of 40 g of dry soil to the cup. The spot application was made by placement of 20 g of soil in the cups, followed by 3 mL of water. The spot treatment was applied to the center of the soil surface as a single 0.4 mL droplet, followed by addition of 20 g of dry soil and then with 3 mL of water. The spent molasses had an analysis (on an "as-received" basis) of 332 g $kg^{-1}$ water, 134 g $kg^{-1}$ crude protein, 196 g $kg^{-1}$ ash, and 176 g $kg^{-1}$ total sugars. The spent molasses contained 58 mg $kg^{-1}$ Fe. At a rate of 0.2 g $cup^{-1}$, the rate of Fe added to the soil in the spent molasses or molasses was only about 0.2 to 0.3 mg $kg^{-1}$ of soil. The processes used to desugarize molasses, and the nature of the spent molasses by-product, are described by Perschak (1998).

### Field Studies.

Field experiments were established at five locations in eastern North Dakota, on soils with a history of producing Fe deficiency chlorosis in soybean. Chemical characteristics of the sites are shown in Table 1. The experimental design was three treatments with three replicates in a randomized complete block design. Treatments were a control, 45 kg ha$^{-1}$ of ferrous sulfate monohydrate (14 kg Fe ha$^{-1}$), and 45 kg ha$^{-1}$ of ferrous sulfate plus 280 kg ha$^{-1}$ of sugarbeet molasses. Individual plot size was 2 x 10 m, except at Galesburg where the plot size was 4 x 10 m. The treatments were applied in October, 1999, to untilled wheat straw, except at the Galesburg site where the crop residue was corn (*Zea mays* L.) stover. The treatments were dissolved in water, sprayed on the crop residues, allowed to dry for 1 to 2 days, and incorporated by rototilling at all sites, except for Galesburg, where the corn stalks were moldboard plowed.

Soil samples were taken at one and seven months after application, November 1999 and May 2000, respectively. The Galesburg site was not sampled on either date because the treatments were plowed down, not mixed with the topsoil. Eight 10-cm diameter bucket auger cores were taken per plot to a 15-cm depth, according to a predetermined sampling plan. The entire sample was air-dried and crushed to pass a 2-mm sieve. All materials, primarily crop residues, passing over the sieve were retained, ground separately (< 0.5 mm), and then mixed back into the sieved sample. After thorough mixing, duplicate samples were analyzed for DTPA-extractable Fe, as previously described.

Given the variable nature of Fe chlorosis in the field, the spring soil samples were also used to grow plants in the greenhouse. One kg of white sand was mixed with solutions providing 75 mg N as calcium nitrate, 25 mg N as magnesium nitrate, and 50 mg P as dibasic potassium phosphate. The moistened sand was then mixed with 1 kg of dry soil, and placed in 2 L closed-bottom pot. Two pots from each field plot were prepared. One pot was kept at 150 g kg$^{-1}$ water content, and the other at 200 g kg$^{-1}$ water content, to induce variable levels of Fe chlorosis. Eight 'Glacier' soybean seeds were planted per pot, and later thinned to the four strongest seedlings. The pots were weighed daily and water added to compensate for evaporation. The relative chlorophyll contents of the first and second trifoliolate leaves were determined with a Minolta SPAD meter.

The field sites were tilled and planted to Glacier soybean, planted at 300,000 seed ha$^{-1}$ on 30-cm centers. Planting date was 19 May 2000 for all sites. Chlorosis was rated visually at the 2 to 3 and 5 to 6 trifoliolate stages, using a 1 to 5 scale, where 1=no chlorosis, 2=slight chlorosis on the upper leaves, but without prominent interveinal chlorosis (the veinal and interveinal tissues were slightly chlorotic and the same color), 3=distinct interveinal chlorosis of the upper leaves, but without obvious reduction of plant height or upper leaf size, and no necrosis, 4=distinct interveinal chlorosis, with obvious reduction in growth, leaf size, or some necrosis, and 5=severe interveinal chlorosis and necrosis, with damage to the growing point, or dead plants. Ratings were made ± 0.5 chlorosis unit. At crop maturity, the plots were trimmed to a 6-m length and seed yield determined.

## RESULTS AND DISCUSSION

### Laboratory Experiment 1.

The effect of several plant materials on DTPA-extractable Fe in the Ulen soil is found in Table 2. Incubation of the soils with the plant materials, in the absence of ferrous sulfate, had little effect on Fe solubility. Also, ferrous sulfate, applied without plant materials, was strongly adsorbed by the soil. Little of the original Fe application could be solubilized by DTPA, even after 1 week of incubation. By contrast, there

**Table 2.** Effect of ferrous sulfate and various plant materials on the concentration of DTPA-extractable Fe in an alkaline soil. Laboratory Experiment 1.

| Ferrous sulfate | Plant material | Length of incubation, weeks 1 | 2 | 4 |
|---|---|---|---|---|
| mg Fe kg$^{-1}$ | | ---------- mg Fe kg$^{-1}$ ---------- | | |
| 0 | None | 4.4 | 4.7 | 3.7 |
| | Alfalfa | 4.5 | 4.5 | 4.0 |
| | Pigweed | 4.4 | 4.7 | 3.9 |
| | Soybean meal | 4.9 | 5.4 | 4.5 |
| 200 | None | 7.7 | 7.2 | 6.2 |
| | Alfalfa | 27.2 | 20.3 | 14.3 |
| | Pigweed | 24.2 | 21.1 | 14.6 |
| | Soybean meal | 23.8 | 15.7 | 12.1 |
| Significance of F | | | | |
| Ferrous sulfate | | ** | ** | ** |
| Plant material | | ** | ** | ** |
| FS x PM | | ** | ** | ** |
| LSD (0.05) | | 3.4 | 1.8 | 2.4 |

**, F test significant at the 0.01 level.

was a strong effect of the plant materials on Fe solubility when applied with ferrous sulfate, increasing Fe solubility by at least 2 to 3-fold over ferrous sulfate applied without added plant materials. The three plant materials tested were similar in their ability to increase Fe solubility.

The observation that Fe solubility in soil is increased when the Fe is applied to crop residues before application to soil may be explained in two ways. The first explanation is that the mechanisms of adsorption of Fe to crop residues result in greater Fe solubilities compared to precipitation of Fe into inorganic oxides. The second explanation is that Fe solubility is improved with microbial production of compounds like citrates, oxylates, and siderophores. Both iron-organic matter adsorption reactions and the nature of microbial siderophores have been reviewed by Stevenson (1991).

### Laboratory Experiment 2.

The effect of ferrous sulfate and several plant materials on DTPA-extractable Fe in the Ulen soil is shown in Table 3. The addition of ferrous sulfate in the absence of a plant material increased Fe solubility over the control, but the magnitude of the increase declined with length of incubation. Application of Fe with the various plant materials led to additional increases in Fe solubility over Fe added alone. The effect was most dramatic for ferrous sulfate added with beet molasses. Averaged across rate of plant material and time of incubation, the concentration of DTPA-extractable Fe was 3.9 mg $kg^{-1}$ for the control, 8.0 mg $kg^{-1}$ for ferrous sulfate applied alone, 9.6 to 11.0 mg $kg^{-1}$ for ferrous sulfate applied with the three dried plant materials, but 20.8 mg $kg^{-1}$ for ferrous sulfate applied with beet molasses.

Ferrous sulfate solutions in water are not stable, and slowly react with oxygen, become cloudy, and form an iron oxide precipitate. Ferrous sulfate solutions are often stabilized with other compounds like citrates or lignosulfonates to make commercial liquid fertilizers. We found that ferrous sulfate-beet molasses solutions were stable and had good handling characteristics. Informal reports from the micronutrient industry suggest that lignosulfonates are sometimes in short supply. More research is needed to determine if ferrous sulfate-beet molasses solutions would make acceptable fertilizers for soil or foliar application, perhaps as a potential substitute for Fe-lignosulfonate.

### Laboratory Experiment 3.

The effect of plant material, ferrous sulfate, and ammonium sulfate on DTPA-extractable Fe in a Ulen soil is shown in Table 4. In the absence of ferrous sulfate, Fe solubility tended to be lower in the presence

**Table 3**. Effect of ferrous sulfate and various rates of different plant materials on the concentration of DTPA-extractable Fe in an alkaline soil. Laboratory Experiment 2.

| Ferrous sulfate | Plant material Source | Rate | Length of incubation, weeks 1 | 2 | 4 | 8 |
|---|---|---|---|---|---|---|
| mg Fe kg$^{-1}$ | | g kg$^{-1}$ | ———mg Fe kg$^{-1}$ ——— | | | |
| 0 | None | — | 4.7 | 4.2 | 3.7 | 3.1 |
| 100 | None | — | 10.9 | 8.7 | 7.3 | 5.1 |
| 100 | Wheat straw | 5 | 12.5 | 12.5 | 8.1 | 7.0 |
| | | 10 | 13.7 | 11.4 | 9.0 | 8.0 |
| | | 20 | 14.6 | 11.7 | 8.6 | 8.8 |
| 100 | Sunflower hulls | 5 | 11.0 | 9.6 | 7.8 | 6.7 |
| | | 10 | 14.3 | 12.4 | 9.5 | 8.7 |
| | | 20 | 17.3 | 14.4 | 11.1 | 9.5 |
| 100 | Beet pulp | 5 | 10.9 | 8.1 | 6.5 | 4.3 |
| | | 10 | 12.8 | 10.3 | 8.4 | 5.6 |
| | | 20 | 16.9 | 13.6 | 10.8 | 7.1 |
| 100 | Beet molasses | 5 | 28.1 | 20.8 | 14.8 | 8.6 |
| | | 10 | 31.6 | 24.6 | 17.6 | 9.9 |
| | | 20 | 35.8 | 27.4 | 19.4 | 11.0 |
| Significance of F | | | | | | |
| Treatment | | | ** | ** | ** | ** |
| LSD (0.05) | | | 3.8 | 3.1 | 2.3 | 2.4 |

**, F test significant at the 0.01 level.

**Table 4**. Effect of different plant materials, ferrous sulfate, and ammonium sulfate on the concentration of DTPA-extractable Fe in an alkaline soil. Laboratory Experiment 3.

| Plant material | Ferrous sulfate | Ammonium sulfate | Length of incubation, weeks 1 | 2 | 4 | 8 |
|---|---|---|---|---|---|---|
| | | | mg Fe kg$^{-1}$ | | | |
| None | Minus | Minus | 4.8 | 3.8 | 3.4 | 3.1 |
| | Minus | Plus | 4.9 | 3.8 | 3.1 | 2.9 |
| | Plus | Minus | 9.2 | 7.6 | 5.9 | 5.4 |
| | Plus | Plus | 10.8 | 7.3 | 6.3 | 5.3 |
| Straw | Minus | Minus | 3.4 | 2.5 | 2.2 | 2.0 |
| | Minus | Plus | 4.4 | 3.0 | 3.0 | 2.2 |
| | Plus | Minus | 13.5 | 9.6 | 8.1 | 7.1 |
| | Plus | Plus | 13.4 | 8.6 | 7.6 | 7.5 |
| Molasses | Minus | Minus | 4.7 | 3.4 | 3.2 | 3.4 |
| | Minus | Plus | 5.4 | 3.7 | 3.2 | 3.4 |
| | Plus | Minus | 23.4 | 13.2 | 10.6 | 9.8 |
| | Plus | Plus | 21.6 | 13.0 | 10.5 | 9.9 |
| Significance of F | | | | | | |
| Plant material | | | ** | ** | ** | ** |
| Ferrous sulfate | | | ** | ** | ** | ** |
| Ammonium sulfate | | | NS | NS | NS | NS |
| PM x FS | | | ** | ** | ** | ** |
| PM x AS | | | + | NS | NS | + |
| FS x AS | | | NS | + | NS | NS |
| PM x FS x AS | | | * | NS | + | NS |
| LSD (0.05) | | | 1.0 | 0.9 | 0.7 | 0.3 |

+, *, **, F test significant at the 0.10, 0.05, and 0.01 levels, respectively. NS, not significant at the 0.1 level.

of straw than without. Applying ferrous sulfate without a plant material increased DTPA-extractable Fe, but Fe solubility was consistently greater when the ferrous sulfate was mixed with straw or molasses before application to soil. As in Experiment 2, application of ferrous sulfate with molasses led to higher extractable Fe than when ferrous sulfate was applied with wheat straw. Ammonium sulfate had no effect on DTPA-extractable Fe. Ammonium sulfate should have aided in microbial decomposition of the two low protein plant materials tested, but apparently this did not influence Fe solubility.

**Laboratory Experiment 4.**

The effect of ferrous sulfate, molasses, spent molasses, and method of application on DTPA-extractable Fe is shown in Table 5. Solubility of Fe from ferrous sulfate, without molasses or spent molasses, was influenced by the method of application. Mixing the ferrous sulfate with the bulk of the soil gave greater residual DTPA-extractable Fe than when the same amount of Fe was applied in a spot application. Spot, or band, application is thought to improve the residual solubility of many nutrients compared to applications mixed with the bulk of the soil. The opposite was observed for ferrous sulfate. Perhaps with a mixed application, there is more contact with soil humic compounds that hold the Fe in a form more easily extracted by DTPA, than with a spot application.

Both molasses and spent molasses increased Fe solubility over ferrous sulfate applied alone. Molasses and spent molasses increased Fe solubility similarly, even though the spent molasses product had a greater water content. This represents a possible new use for spent molasses. Spent molasses currently has little commercial value (about 10 $US per 1000 kg at the factory), and its only identified use is as an animal feed supplement (Perschak, 1998).

Preliminary observations suggest that ferrous sulfate-spent molasses solutions are also stable and store well. Further research is needed to determine if ferrous sulfate-spent molasses solutions might be an acceptable alternative to Fe-lignosulfonate in the fertilizer market for foliar or soil application.

**Field Studies.**

The ferrous sulfate solution or ferrous sulfate plus molasses solution were applied to crop residues in October, 1999, and soil samples taken in November, 1999 and May, 2000. With the fall soil samples (Table 6), results were very consistent. Application of ferrous sulfate to wheat straw increased DTPA-extractable Fe levels in the soil over the control at

**Table 5**. Effect of ferrous sulfate, plant material, and method of application on the concentration of DTPA-extractable Fe in an alkaline soil. Laboratory Experiment 4.

| Ferrous sulfate | Plant material † | Applic. method | Length of incubation, weeks | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 4 | 6 | 8 |
| | | | mg Fe $kg^{-1}$ | | | | |
| 0 | None | — | 5.2 | 4.2 | 3.7 | 3.1 | 3.1 |
| 100 | None | Mixed | 13.7 | 11.8 | 9.0 | 7.9 | 6.7 |
| | Mol | Mixed | 25.4 | 20.3 | 15.1 | 12.0 | 11.1 |
| | SM | Mixed | 26.4 | 21.1 | 13.7 | 12.2 | 7.8 |
| 100 | None | Spot | 9.7 | 8.0 | 6.2 | 5.4 | 5.2 |
| | Mol | Spot | 26.7 | 18.9 | 13.7 | 10.1 | 9.6 |
| | SM | Spot | 30.6 | 23.1 | 16.1 | 11.9 | 11.0 |
| Significance of F<br>Treatment | | | ** | ** | ** | ** | ** |
| LSD (0.05) | | | 3.0 | 2.0 | 2.6 | 1.3 | 1.2 |

† Mol=beet molasses, SM=spent molasses.
**, F test significant at the 0.01 level.

**Table 6.** Effect of ferrous sulfate and beet molasses applied to wheat stubble in October, 1999, on the concentration of DTPA-extractable Fe in November, 1999 and May, 2000. Four field sites, eastern ND.

| Ferrous sulfate | Molasses | Site | | | |
|---|---|---|---|---|---|
| | | Argusville | Arthur | Ayr | Casselton |
| kg ha$^{-1}$ | kg ha$^{-1}$ | mg Fe kg$^{-1}$ | | | |
| **Fall samples** | | | | | |
| 0 | 0 | 7.9 | 6.7 | 7.2 | 8.5 |
| 45 | 0 | 8.8 | 7.2 | 8.5 | 9.6 |
| 45 | 280 | 9.0 | 8.2 | 9.3 | 11.3 |
| Significance of F | | | | | |
| Treatment | | ** | ** | ** | ** |
| LSD (0.05) | | 0.6 | 0.4 | 0.5 | 0.7 |
| **Spring samples** | | | | | |
| 0 | 0 | 4.3 | 3.6 | 3.4 | 6.0 |
| 45 | 0 | 3.0 | 4.0 | 5.1 | 6.6 |
| 45 | 280 | 4.7 | 6.3 | 5.3 | 6.9 |
| Significance of F | | | | | |
| Treatment | | ** | ** | ** | ** |
| LSD (0.05) | | 0.5 | 0.4 | 0.6 | 0.4 |

**, F test significant at the 0.01 level.

all four sites. The effect was even larger when ferrous sulfate plus molasses was sprayed on wheat straw. With the spring soil samples, results at Argusville were inconsistent. The soil treated with the ferrous sulfate had less DTPA-extractable Fe than the control. At the other three sites, spraying wheat straw with ferrous sulfate increased DTPA-extractable Fe over the control. At all four sites, the greatest extractable Fe levels were found when ferrous sulfate plus molasses was sprayed on stubble before tillage.

Spring soil samples were taken to the greenhouse and used to grow soybean plants. Normal soybean leaves are generally associated with chlorophyll levels above 30 as measured with a SPAD meter, while severe chlorosis is associated with readings below 15. Soil samples from the Argusville and Casselton sites produced plants with the greatest chlorophyll levels (least chlorosis) and no effect of the fall-applied Fe treatments was observed (Table 7).

**Table 7**. Effect of ferrous sulfate and molasses applied to wheat stubble in the field in October, 1999, on chlorophyll content of soybean leaves grown in the greenhouse. Average of first and second trifoliolate leaves. Soil samples taken in May, 2000, used to grow plants in the greenhouse. Two water levels were used to induce differing degrees of chlorosis severity.

| Ferrous sulfate | Molasses | Water content | Site<br>Argusville | <br>Arthur | <br>Ayr | <br>Casselton |
|---|---|---|---|---|---|---|
| kg ha$^{-1}$ | kg ha$^{-1}$ | g kg$^{-1}$ | — Relative chlorophyll content † — | | | |
| 0 | 0 | 150 | 29.0 | 24.3 | 24.9 | 30.0 |
| | | 200 | 27.4 | 9.1 | 17.4 | 28.8 |
| 45 | 0 | 150 | 27.6 | 20.9 | 28.1 | 31.1 |
| | | 200 | 23.3 | 15.0 | 23.3 | 28.6 |
| 45 | 280 | 150 | 26.8 | 24.3 | 26.7 | 30.5 |
| | | 200 | 25.8 | 15.3 | 23.2 | 28.3 |
| Significance of F | | | | | | |
| Treatment | | | NS | NS | * | NS |
| Water | | | NS | ** | ** | ** |
| Treatment x Water | | | NS | NS | NS | NS |
| LSD (0.05) | | | 8.3 | 8.5 | 4.6 | 1.6 |

† Relative chlorophyll content, as indicated by a Minolta SPAD meter.
*, **, F test significant at the 0.05, and 0.01 levels, respectively. NS, not significant at the 0.1 level.

Chlorosis was most severe with plants grown on soil from the Arthur and Ayr sites. Chlorosis at the soil high water content was reduced by both fall-applied Fe treatments, with soil from the Arthur site. The clearest effect of treatment on plant chlorosis was from soil from the Ayr site. Both fall-applied ferrous sulfate and ferrous sulfate plus molasses increased leaf chlorophyll levels. At the soil high water level, with soil from both the Arthur and Ayr sites, the fall-applied Fe treatments increased chlorophyll levels by about 6 SPAD units. This is an increase of similar magnitude as one might obtain with the selection of a more resistant cultivar or planting at a greater seeding rate (Goos and Johnson, 2000b, Goos and Johnson, 2001).

The effect of fall-applied ferrous sulfate and molasses on chlorosis levels in the field is shown in Table 8. The Arthur site was abandoned after heavy June rains caused ponding and loss of most of the experiment. Chlorosis developed early at all four sites, but varying degrees

**Table 8**. Effect of fall-applied ferrous sulfate and molasses on the severity of iron deficiency chlorosis in soybean the following year, 2-3 and 5-6 trifoliolate stages of growth. Four field sites, eastern ND, 2000.

| Ferrous Sulfate | Molasses | Site | | | |
|---|---|---|---|---|---|
| | | Argusville | Ayr | Casselton | Galesburg |
| kg ha$^{-1}$ | kg ha$^{-1}$ | Chlorosis score † | | | |
| **2-3 trifoliolate stage** | | | | | |
| 0 | 0 | 3.2 | 3.3 | 3.3 | 3.2 |
| 45 | 0 | 3.0 | 2.8 | 3.2 | 3.3 |
| 45 | 280 | 3.2 | 2.8 | 3.2 | 3.3 |
| Significance of F<br>Treatment | | NS | NS | NS | NS |
| LSD (0.05) | | 0.8 | 0.8 | 0.4 | 0.8 |
| **5-6 trifoliolate stage** | | | | | |
| 0 | 0 | 2.7 | 2.8 | 2.7 | 2.7 |
| 45 | 0 | 2.5 | 2.5 | 2.7 | 2.7 |
| 45 | 280 | 2.7 | 2.3 | 2.8 | 2.7 |
| Significance of F<br>Treatment | | NS | NS | NS | NS |
| LSD (0.05) | | 0.8 | 0.6 | 0.4 | 0.6 |

† 1=no chlorosis, 5=severe chlorosis, see text.
NS, F test not significant at the 0.1 level

of recovery was observed at all sites. No significant effects of treatments on chlorosis were observed at any site at either the 2 to 3 or 5 to 6 trifoliolate stages of growth. The only site giving a trend for reduced chlorosis was Ayr, where the chlorosis scores of both ratings were reduced by about 0.5. Although not statistically significant, the controls appeared more chlorotic in two of three replicates at the Ayr site. Seed yields indicated that the plants recovered from early chlorosis all sites but Ayr. No effects of treatment on seed yield were observed (Table 9).

**Table 9.** Effect of fall-applied ferrous sulfate and molasses on seed yield of soybean the following year. Four field sites, eastern ND, 2000.

| Ferrous sulfate | Molasses | Site<br>Argusville | <br>Ayr | <br>Casselton | <br>Galesburg |
|---|---|---|---|---|---|
| kg ha$^{-1}$ | kg ha$^{-1}$ | kg ha$^{-1}$ | | | |
| 0 | 0 | 3102 | 1556 | 3558 | 2973 |
| 45 | 0 | 3309 | 1855 | 3888 | 2745 |
| 45 | 280 | 3200 | 1679 | 3729 | 2797 |
| Significance of F | | | | | |
| Treatment | | NS | NS | NS | NS |
| LSD (0.05) | | 83 | 169 | 98 | 236 |

NS, F test not significant at the 0.1 level

The application of ferrous sulfate or ferrous sulfate plus molasses in the field consistently increased soil test Fe concentrations, and showed some beneficial effects on chlorosis in the greenhouse, but the effects on chlorosis were disappointing in the field. Perhaps the time interval between application (October) and the development of chlorosis (June) was too long, or perhaps a more localized application (with or near the seed) should have been performed. Further studies are needed to determine if other methods of application of ferrous sulfate-molasses or ferrous sulfate-spent molasses solutions (e.g., spring incorporated, banded with the seed, foliar) would be effective in reducing chlorosis in soybean.

**Conclusions.**

1. Adding ferrous sulfate to plant materials before incorporation into soil improved DTPA-extractable Fe concentrations over application of ferrous sulfate alone.

2. Two sugarbeet by-products, regular molasses and spent molasses, were shown to be superior to other plant materials for increasing the solubility of Fe from ferrous sulfate. Molasses and spent molasses were similar in their ability to increase Fe solubility from ferrous sulfate.
3. Amending plant materials with both ammonium sulfate and ferrous sulfate had no effect on Fe solubility above that observed when the plant materials were amended with ferrous sulfate alone.
4. In the field, the highest levels of DTPA-extractable Fe in soil were observed with the ferrous sulfate plus molasses treatment.
5. Chlorosis tended to be reduced at only one of four field sites, and grain yields of soybean were not increased by fall applied Fe treatments.
6. Further studies are needed to evaluate ferrous sulfate-molasses or ferrous sulfate-spent molasses solutions with other methods and timing of application.

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